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Takeoff temperatures in *Melitaea cinxia* butterflies from latitudinal and elevational range limits: a potential adaptation to solar irradiance

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1 **Takeoff temperatures in *Melitaea cinxia* butterflies from latitudinal and elevational range**
2 **limits: a potential adaptation to solar irradiance**

3

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15

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17 climate change adaptation, solar irradiance.

18 **Running title:** Takeoff temperatures in butterflies

19

20

21 **Abstract**

22

23 1. We provide evidence that a heliophilic butterfly, the Glanville Fritillary (*Melitaea cinxia*) has
24 adapted differently to environmental variation across latitudes and elevations.

25

26 2. In cool air, basking *M. cinxia* orient themselves perpendicular to the sun's rays to gain heat and
27 take off. During flight, solar heating is reduced because orientation perpendicular to the sun is no
28 longer possible and convective cooling occurs. Consequently, *M. cinxia* have been shown to
29 suffer net heat loss in flight, even in full sunshine. When flight duration is restricted in this way,
30 the takeoff temperature becomes an important thermal adaptation.

31

32 3. Using a thermal imaging camera, we measured takeoff temperatures in our experimental
33 butterflies. Butterflies from the northern range limit in Finland took flight at slightly hotter
34 temperatures than butterflies from the southern limit in Spain, and much hotter than butterflies
35 from the elevational limit (1900-2300 m) in the French Alps. Butterflies from low-elevation
36 populations in southern France also took off much hotter than the nearby Alpine population.

37

38 4. These results suggest that influences of elevation differ from those of latitude in more respects
39 than ambient temperature. Values of solar irradiance in the butterflies' flight season in each region
40 show that insects from the coolest habitats, Finland and Alps, experienced similar solar irradiance
41 during basking, but that Finns experienced much lower irradiance in flight. This difference may
42 have favored Finnish butterflies evolving higher takeoff temperatures than Alpine butterflies that
43 also flew in cool air but benefited from more intense radiant energy after takeoff.

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1. Introduction

66 As projections of species' responses to climate change acquire greater urgency (Urban 2015), they
67 are also acquiring greater complexity. Nadeau et al. (2017) consider how spatial and temporal
68 variability of climates past and present might influence both dispersal and thermal tolerance.
69 Badik et al. (2015) examine how within-year variation in timing and intensity of precipitation
70 predicted changes in species richness across an elevational transect. Other models derive
71 predictions by combining experimental measurements of physiological responses with climate
72 envelopes of current distributions (Kearney & Porter 2009, Araujo et al. 2013, Sunday et al.
73 2014). Despite these increasingly sophisticated approaches to climate data, Species Distribution
74 Models (SDM's) still calculate each species' climate space from bioclimatic variables and use this
75 information to predict latitudinal and elevational range shifts (e.g. Jueterbock et al. 2016).

76

77 In some cases, regional temperature changes suffice to account quantitatively for observed range
78 shifts (Parmesan 1996, Crozier 2004). In other examples, changes in precipitation are more
79 important than temperature; for example, they account for range shifts of North American trees,
80 explaining general trends for westward shifts of angiosperms (Fei et al. 2017). Where temperature
81 is the most important factor, projections of shifts in range, abundance, or demography generally
82 carry the implicit assumption that responses to changes of ambient temperature will be similar
83 whether those changes are measured along latitudinal or elevational gradients (Parmesan 1996,
84 DeVicor et al. 2012). However, operating body temperatures of heliophilic poikilotherms are
85 strongly affected by solar irradiance, which varies differently with elevation and latitude, as we
86 shall illustrate. We might therefore expect that temperature data alone will fail to explain how
87 thermal adaptations of these species vary across species' ranges, and that influences of elevation
88 and latitude might differ.

89

90 Here, we begin to address this question by seeking local adaptation of an easily-measured thermal
91 adaptation, body temperature at spontaneous takeoff, in a heliophilic insect, the Glanville
92 Fritillary butterfly (*Melitaea cinxia*) sampled from its elevational and latitudinal range extremes.

93

94 The ability of heliophilic insects to regulate body temperature allows them to be active in places
95 and at times from which they would otherwise be excluded (Heinrich, 1995), with the result that
96 they can be found at higher altitudes and latitudes than might be expected of any thermophilic
97 poikilotherm. In Sunday et al's (2014) meta-analysis of cold tolerance across all poikilotherms,
98 studies of insects extended to higher latitudes (>60 degrees) than those of reptiles or amphibians.

99 The **Dingy (or improbable)** fritillary (*Boloria improba*) for example, has its equatorial range limit
100 in northern Finland and does not occur at low elevation where the Fennoscandian mainland meets
101 the Arctic Ocean (Lafranchis 2004). Even more improbably, five butterfly species in three
102 different families were recorded as residents at Lake Hazen in Nunavut, **northern Canada**, at
103 latitude 81.5°N (Kevan 1972).

104

105 Thoracic temperatures of arctic-alpine and temperate zone butterflies must exceed a lower
106 threshold for performance of controlled flight and are crucial for dispersal ability, predator
107 avoidance, foraging, mate finding, fecundity and oviposition (Watt 1968, Kingsolver & Watt
108 1983, Kemp & Krockenberger 2002, Berwaerts et al. 2008, Velde et al. 2011). When air
109 temperatures are cool, both temperate zone and arctic-alpine butterflies cycle through periods of
110 activity and inactivity: in sunshine they bask, thermoregulate, and fly; when a cloud passes across
111 the sun they alight and quickly become dormant. During flight in cool weather, small butterflies,

112 including our study insects, lose heat even in full sunshine and must alight frequently to bask and
113 re-warm (Mattila 2015). In these conditions takeoff temperature, the thoracic temperature at
114 which individuals take flight in the absence of specific flight-inducing stimuli such as a predator
115 or competitor, must strongly affect the overall proportion of time spent flying. Hence, takeoff
116 temperature is a likely target of natural selection associated with climatic variation.

117

118 Interspecific variation in takeoff temperature can be extreme. For example, Neve and Hall (2016)
119 reported that the thoracic temperatures of Australian butterflies at spontaneous takeoff ranged
120 from 13.4°C to 46.3°C. Variation observed among congeners is also substantial: *Colias* in
121 Colorado flew at higher body temperatures than those in Alaska, whereas within Colorado, a low-
122 elevation species flew at higher temperatures than its high-elevation congener (Kingsolver 1983,
123 Heinrich 1993, MacLean et al. 2016).

124

125 *M. cinxia* is well-known ecologically, behaviorally and genetically (Hanski 2011). Previous
126 studies of intraspecific variation in takeoff temperature of this species have examined plastic
127 responses of Finnish insects to the environment and within-population differences among
128 genotypes and between sexes (Saastamoinen & Hanski 2008, Mattila 2015). Here, we
129 complement these studies by reporting takeoff temperatures at the elevational and latitudinal
130 extremes of the species' range, with Finland included as the northern range limit.

131

132 *M. cinxia* is non-migratory, with levels of gene flow and genetic variation that permit adaptation
133 to local climatic conditions. Even within the relatively small area (c.50x40km) of the intensively
134 studied Finnish metapopulation of *M. cinxia*, habitat patches varied in heat-shock protein and in

135 phosphoglucose isomerase (Pgi) genotype. Hsp70 genotype was variable and associated with
136 takeoff temperature, while Pgi variation interacted with temperature to affect flight metabolic rate,
137 body temperature in flight, and dispersal likelihood (Niitepold et al. 2009, Niitepold 2010, Mattila
138 2015).

139
140 On a larger scale, the most recent common ancestor of populations at the species' latitudinal range
141 limits existed at least 500,000 generations ago (Wahlberg & Saccheri 2007). Given that local
142 adaptation to climate can apparently occur within the Åland Islands, we have strong expectation
143 that these range-limit populations should differ in traits that adapt them to local climate. This
144 expectation is fulfilled: traits relevant to climate adaptation, such as the constitutive level of heat-
145 shock protein Hsp21.4 (Advani et al. 2016) and frequency of alleles affecting tracheal
146 development and oxygen delivery (Marden et al. 2013), do differ between populations at the
147 species' latitudinal range limits.

148

149 **2. Materials and Methods**

150

151 At low elevations in Europe, *M. cinxia* is distributed between approximately 41.8°N in Catalunya
152 in northern Spain and 60.2°N in the Åland Islands in southern Finland (Lafranchis 2004); it is also
153 found at high elevations further south, as far south as the Atlas Mountains in Morocco. The
154 elevational range of the butterfly at mid-latitude is from sea level to 2350m in the Alps
155 (LaFranchis 2004), with occasional individuals at higher elevations.

156

157 Wild-caught female *M. cinxia* provided eggs and additional egg clutches were found in the field.
158 We considered each egg clutch to be an independent sample from its population, and analysis
159 assumes this independence. While we could not control for maternal effects, all individuals tested
160 underwent development under near-identical conditions at the University of Texas at Austin,
161 albeit at different times given the length of this study. This included feeding larvae on a
162 combination of *Plantago lanceolata*, *Plantago alpina* and *Veronica spicata*, in petri dishes, at
163 room temperature (22°C), under growth lights. Winter diapause lasted 3-4 months in a climate
164 controlled cold room (4°C). Once the adult butterflies eclosed, they were kept in separate cages
165 and were fed daily with a honey and water solution.

166

167 We tested individuals from five geographically separate regions representing the latitudinal and
168 elevational extremes of the species' range.

169 To make an elevational comparison, we sampled two regions:

170 1) The elevational limit at 1900-2350m in the French Alps (2 populations, 12 families, 45
171 individuals)

172 2) Low-elevation southern French sites at 50-250 m elevation, around 180km from the Alpine
173 sites (4 populations, 9 families, 13 individuals).

174

175 To compare insects from different latitudes and similar (low) elevation we sampled three regions:

176 1) The low-elevation southern range limit in Catalunya (Spain) (4 populations, 19 families, 38
177 individuals)

178 2) The northern range limit in the Åland Islands (Finland) (4 populations, 8 families, 24

179 individuals - but populations were lumped; population identity was not retained with each family)

180 3) The northern range limit in the Isle of Wight (UK) (1 population, 11 families, 15 individuals).

181

182 These regions encompass the climate extremes that *M. cinxia* experiences. Table 1 shows

183 geographical positions of the study populations within the regions, their elevations and relevant

184 data on flight dates and climatic variables.

185

186 Alpine *M. cinxia* are univoltine (one generation per year), flying in June and early July, while the

187 low-elevation southern French butterflies are bivoltine, usually flying in April-May and again in

188 July (table 1). The elevational comparison undertaken here was between Alpine and second-

189 generation French butterflies that normally fly at approximately the same time of year. The

190 latitudinal comparison was between insects that would normally fly in April/May in Spain and

191 June in Finland (table 1), so only by raising them in the laboratory were we able to test them side-

192 by-side.

193

194 2.1. Thermal images

195

196 To capture thermal images, we used a camera (MikroScan 7515 Thermal Imager) that visualizes

197 infrared (IR) energy emitted by an object as a color thermal image. The camera also incorporates a

198 background compensation feature, to remove errors caused by IR radiation from background

199 objects. One manually sets the camera according to the emissivity of the subject, which is the

200 extent to which it reflects, absorbs and transmits IR energy. As part of the calibration, the camera

201 allows the operator to establish the emissivity of a single point within the field of view. The

202 emissivity of the butterfly thorax was determined as 0.95, consistent with the value found by

203 Palmer et al. (2004), and the exact value used for *M. cinxia* by Mattila (2015). We set the camera
204 to 0.95 emissivity for the entire set of experiments. We also kept the ambient compensation
205 settings of the camera at a constant temperature of 20°C and an object distance of 35 cm.

206

207 We might expect that heating of the flight muscles during basking would be delayed relative to
208 external heating recorded by the thermal camera. To assess this possibility, we used a small
209 sample of individuals (n = 4) to investigate the relationship between the thermal images of the
210 thorax and the internal temperature of the butterfly by inserting a temperature probe (MT-29/1B
211 Insect Probe, Type T, Copper-Constantan Thermocouple) into the side of the thorax. The probe
212 provided continuous measurements of the internal thoracic temperature of the butterfly as it
213 warmed up. We measured internal thoracic temperatures at the same time that each thermal image
214 was taken.

215

216 *2.2. Testing of takeoff temperature*

217

218 Trials were conducted in a climate-controlled greenhouse in full sun, with ambient temperature
219 close to 20°C. Only one butterfly at a time was tested. It was observed anecdotally that recent
220 feeding reduced the tendency to fly, hence we refrained from testing within three hours after
221 feeding. Prior to each trial the test butterfly was cooled in the shade next to an air conditioning
222 vent. It was then taken out of its cage and allowed to bask in full sunlight on a white card. The
223 white card was chosen to minimize **absorption of heat by the surface**, and the card was also cooled
224 so as to not contribute to insect warming. Typically, a butterfly would start the test with wings
225 closed, and then after a few seconds in the sun spread its wings into a dorsal basking posture,

226 orient itself such that the plane of solar radiation was perpendicular to its body, gradually warm
227 up, and then either take spontaneous flight or close its wings, preventing further heating. Thermal
228 images were taken manually every few seconds.

229

230 Data were included if they met the following criteria during trials (if criteria were not met, the
231 data were excluded):

232

233 1) The butterfly began by basking in constant direct sunlight and continued to do so until just
234 before takeoff.

235 2) The butterfly stayed on the same spot on the card, from the time it was placed there until the
236 time it took off. Behaviors violating this requirement included flapping of the wings while
237 warming up, walking around the card while warming up, or closing the wings above the body
238 after heating up instead of taking off.

239 3) A usable thermal image was captured no more than 5 seconds before the butterfly took off (this
240 was the image used for final analysis of takeoff temperature).

241

242 *2.3. Thermal image analysis*

243

244 Thermal image analysis was conducted using Mikrospec 4.0 software. This program divides the
245 thermal image into a series of pixels, with each pixel assigned a temperature (figure 1). We

246 obtained two measures of thoracic temperature at takeoff. First, our "multipixel" value was an

247 average temperature of as many pixels as possible covering the thorax. This value was calculated

248 from a square grid of pixels such as that shown in figure 1 (black square), typically varying from 9

249 (3x3 pixels) to 16 (4x4 pixels). Our second measure was the temperature of the hottest pixel in the
250 image taken prior to takeoff.

251

252 *2.4. Environmental variables*

253

254 The average daytime temperature during the season(s) when adult butterflies were flying was
255 calculated for each region by taking averages of these data from all the collecting
256 sites/populations within the region. The data used for this calculation were gathered from the
257 European Commission Joint Research Centre (2012). Solar irradiance was calculated for us by
258 John Frederick, using his own algorithm (Frederick & Lubin 1988, Frederick & Liao 2005). For
259 each study site he provided estimates of irradiance received at noon by insects either flying
260 (oriented horizontally) or basking (oriented perpendicular to the sun's rays) (table 1).

261

262 *2.5. Statistical Analyses*

263

264 Statistical analyses were conducted using SPSS (v. 20). Average takeoff temperature per
265 individual was calculated using the data gathered from repeat trials (number of trials varied
266 between 1 to 6 per individual). A nested ANOVA (with individual nested within family, nested
267 within region) was then used to estimate differences among regions. No significant differences
268 were found among populations within a region. Therefore, where regions contained more than one
269 population, different populations within that region were pooled. For pairwise comparisons
270 between regions, the least significant difference (LSD) method was used. A linear regression
271 analysis was conducted for the subset of individuals that had been weighed, plotting individual

272 mass against average take off temperature. ANOVA was used for within-region comparison of the
273 sexes in their thoracic temperatures at takeoff.

274

275 **3. Results**

276

277 *3.1. Relationship between thermal camera data and internal thoracic temperature*

278

279 As basking began, the internal thoracic temperature measured by the thermocouple was cooler
280 than the external thoracic temperature measured by the camera. As the butterfly warmed, the gap
281 between these temperatures became smaller, and eventually the external temperature recorded by
282 the camera was identical or very close to that recorded by the temperature probe (supplemental
283 table 1).

284

285 *3.2. Differences among regions in thoracic takeoff temperature*

286

287 Overall analysis using the "multipixel" measure showed significant heterogeneity of thoracic
288 temperatures at takeoff among the five geographic regions (nested ANOVA: $F=2.921$, $df=4$,
289 $P=0.026$). Pairwise comparisons among the different regions found three significant differences
290 (figure 2). Finnish butterflies took off at hotter temperatures than those from the Alps (LSD:
291 $SE=0.453$, $P=0.016$). Southern French insects had hotter takeoff temperatures than those from
292 nearby Alpine populations (LSD: $SE=0.534$, $P=0.004$), and also hotter than insects from the Isle
293 of Wight (LSD: $SE=0.624$, $P=0.032$).

294

295 Overall analysis using the "hottest pixel" measure (figure 3) also found significant heterogeneity
296 among regions (ANOVA: $F = 2.616$, $df = 4$, $p = 0.042$). The three significant differences found in
297 the "multipixel" measure were again found among "hottest pixels", with the difference between
298 Finland and Alps acquiring greater significance. In addition, two more comparisons were
299 significant with the hottest pixel measure: Finnish butterflies took off with hotter "hottest pixels"
300 than those from both Isle of Wight and Spain (figure 3).

301

302 Unsurprisingly, there is no disagreement between the "hottest pixel" and "multipixel" measures in
303 the direction of interpopulation differences, the difference is in the number of those differences
304 that achieve statistical significance. We know of no biological reason to expect a higher number
305 of interpopulation comparisons to be significant using the "hottest pixel" measure, so this
306 difference may be accidental. In the absence of further knowledge, we place greatest trust in the
307 comparisons that were significant by both measures.

308

309 *3.3. Effects of sex and body mass*

310

311 For individuals for which we had takeoff temperature data as well as mass data, a regression of
312 multipixel takeoff temperature against body mass lacked significance both when the sexes were
313 pooled ($R^2=0.055$, $N=26$ individuals, $F=1.385$, $P=0.251$), and when they were analyzed separately
314 (Females: $R^2=0.04$, $N=11$ individuals, $F=0.377$, $P=0.554$; Males: $R^2=0.005$, $N=15$ individuals,
315 $F=0.070$, $P=0.796$).

316

317 Males and females did not differ significantly in multipixel take off temperature, except in the
318 Finnish (Åland Islands) population (ANOVA: N=24 individuals – 13 females and 11 males;
319 $F=5.271$; $df=1$; $P=0.032$), where the females took off at cooler temperatures than males.

320

321 **4. Discussion**

322

323 *4.1. Differences in takeoff temperature by latitude and elevation*

324

325 Nonmigratory butterfly species such as *M. cinxia* can have ranges encompassing very different
326 climates. Such species might be expected to adapt genetically to their local climates, adaptation
327 that can be illuminated by reciprocal transplants (van Dyck & Holveck 2016) or by comparing
328 individuals raised under the same conditions but sourced from regions in different parts of the
329 species' range. The present study found evidence for local adaptations in a simple but important
330 trait, body temperature at spontaneous takeoff.

331

332 The range of variation in takeoff temperature was not great, which is unsurprising in view of the
333 evolutionary conservatism of thermal traits in general (Kellerman et al. 2012, Buckley &
334 Kingsolver 2012, Araujo et al. 2013). However, takeoff temperature did vary significantly among
335 regions. We found significant regional differences between individuals from southern French sites
336 at the elevational extremes, with cooler takeoff temperatures by the insects from high elevation.
337 However, despite climatic differences between the latitudinal extremes at the seasons when the
338 butterflies fly (table 1), and despite known latitudinal trends in insect thermal tolerances
339 (Lancaster 2016), we found no consistent effect of latitude. Although UK (Isle of Wight) insects

340 did take flight at cooler thoracic temperatures than southern French butterflies, there was no
341 general trend for insects from northern regions to take off at cooler body temperatures than those
342 from southern regions. In particular, Finnish insects took off at slightly higher temperatures than
343 those from the southern range limit in Spain, a difference that achieved significance when we used
344 the "hottest pixel" measure.

345

346 *4.2. Potential explanation for high takeoff temperatures in cool climates*

347

348 If butterflies were unable to evolve efficient flight at low body temperatures, those in cooler
349 climates could be under stronger selection to increase their flight durations by taking off at very
350 high temperatures. Heinrich (1986) observed that *Coenonympha inornata* lost 10°C during each
351 flight, and they extended their flight durations by taking off at much higher temperatures than the
352 minimum needed for active flight. Net heat loss in flight has also been observed in Finnish *M.*
353 *cinxia*, which cooled at mean rates of 0.2-0.4°C/sec when flying in their natural environment
354 (Mattila 2015).

355

356 The hypothesis that takeoff temperatures should be high in cool climates may account for the hot
357 takeoffs of Finnish insects, but not for the cool takeoffs of Alpine butterflies that operate in air
358 averaging only 1-1.5 °C warmer than in Finland (table 1). However, although solar heating is
359 hardly different between Alps and Finland for basking butterflies, the Alpine butterflies receive
360 considerably higher solar irradiance when flying in sunshine (1102 w/m², compared to 927 w/m²
361 in Finland). This high input of radiant heat to flying insects should allow the Alpine butterflies to
362 take off at relatively cool thoracic temperatures. Hence, we begin to suspect that differences in

363 radiant energy may be as important to these insects as differences in ambient air temperature.
364 However, response to solar irradiation will not explain all our results; in particular, the high
365 takeoff temperatures of low-elevation butterflies in southern France are unlikely to be needed to
366 extend flight duration.

367

368 The observation of high takeoff temperatures of Finnish butterflies in the current study is not the
369 only result to show apparently paradoxical inter-site variation of thermal adaptation in butterflies.
370 Vrba et al. (2012) found that overwintering larvae of *Erebia* butterflies from higher elevations
371 were less cold-tolerant than those from lower, the opposite of the simple expectation that denizens
372 of colder climates should be more cold-tolerant. These authors suggested that low elevation larvae
373 may have experienced the most extreme low temperatures if the high mountain insects had been
374 protected by insulating snow. Another interesting avenue of research would be to compare flight
375 behavior in habitats with similar solar irradiance when the sun is shining, but with significant
376 differences in other weather variables (clouds, wind, etc.). Clearly, even though butterflies are
377 relatively well-known poikilotherms, we don't yet have enough information to understand their
378 adaptations to local climate.

379

380 4.3. Effects of sex and mass

381

382 We found no effect of sex on takeoff temperature except in insects from Finland: Finnish males
383 took off at a mean thoracic temperature of 39.8°C, significantly hotter than females at 38.1°C.

384 Mattila (2015), using a protocol that differed from ours in several respects, found a nonsignificant

385 difference in the opposite direction, and much lower mean takeoff temperatures in both sexes
386 (31°C in males and 31.9°C in females).

387
388 Saastamoinen & Hanski (2008), also working with Finnish populations of *M. cinxia*, found a
389 significant difference between the sexes in body surface temperature of butterflies captured during
390 flight, with an average thoracic temperature in males of 28.4°C, and 30.1°C in females. This
391 measure reflects both temperatures at takeoff, rates of cooling in flight and flight durations. It is
392 not explained by sex-specific rates of cooling in flight, since females cooled faster than males
393 (Mattila 2015). We suggest that it would be informative to measure body temperatures
394 immediately after voluntary alighting, i.e., the temperatures at which insects flying in cool air
395 decide to alight and bask, or are forced to do so.

396
397 One might expect larger butterflies to require higher thoracic temperatures at takeoff. This effect
398 was found in a comparison among species (Neve & Hall 2016). Berwaerts and Van Dyck (2004),
399 working with *Pararge aegeria*, found that lighter males with high relative thoracic mass had
400 higher performance than males with a low relative thoracic mass. We however found no effect of
401 mass on takeoff temperature. Mattila (2015), working with Finnish populations of *M. cinxia*,
402 found that large males took off at significantly lower thoracic temperature than small males, but
403 there were no differences amongst females of different mass.

404

405 4.4. Relevance to climate warming

406

407 Acclimation, adaptation, dispersal and behavioral modification all play parts in responses to
408 global warming (Deutsch et al. 2008), as species shift their ranges in latitude and elevation
409 (Parmesan 2006, Socolar et al. 2017, Singer 2017). Detailed studies of physiological mechanisms
410 will improve our projections of climate change impacts (Pörtner & Farrell 2008). However, where
411 it is legal, the most direct evidence to assess coming range shifts can be gleaned from
412 translocating organisms outside their current ranges (Crozier 2004, Pelini et al. 2009). We hope
413 that further studies of *M. cinxia* will be useful in illuminating differences between thermal effects
414 of elevation and latitude that might apply to small poikilotherms in general, and hence contribute
415 to improving predictive ability of the effects of climate change.

416

417 By its very nature, takeoff temperature must be classed as a thermal adaptation. Within the set of
418 populations in our current study, environmental differences associated with elevation seem to
419 have been more important in shaping this adaptation than those associated with latitude. The
420 suggestion from these results is that, in addition to climate, solar irradiance may be influential.
421 Differences among habitats in features other than ambient temperature may prove to be significant
422 drivers of thermal adaptations in poikilotherms.

423

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425

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432

433 **Contribution of Authors**

434

435 All authors participated in collecting and rearing insects, and writing the manuscript. N.K.A. took
436 the thermal images, performed the analyses and wrote the first draft of the MS as part of his Ph.D.
437 dissertation at the University of Texas at Austin

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585

586 Figure/Table Captions

587

588 Figure 1: A sample thermal image captured by the camera. The black square indicates the area
589 used to calculate average thoracic temperature.

590

591 Figure 2: Differences among regions in mean thoracic temperature at takeoff. For each region the
592 numbers in the body of the figure show the number of populations/families/individuals tested.

593 * $p < 0.05$; ** $p < 0.01$.

594

595 Figure 3: Differences among regions in temperature of hottest pixel at takeoff. For each region the
596 numbers in the body of the figure show the number of populations/families/individuals tested.

597 * $p < 0.05$; ** $p < 0.01$.

598

599 Table 1: Environmental variables for the sites used in the study. Geographical locality
600 information; elevation; peak flight date; average daytime temperature at peak flight ($^{\circ}\text{C}$); and,
601 where calculated, noon clear-sky irradiance (w/m^2) at peak flight for flying and basking insects.

602

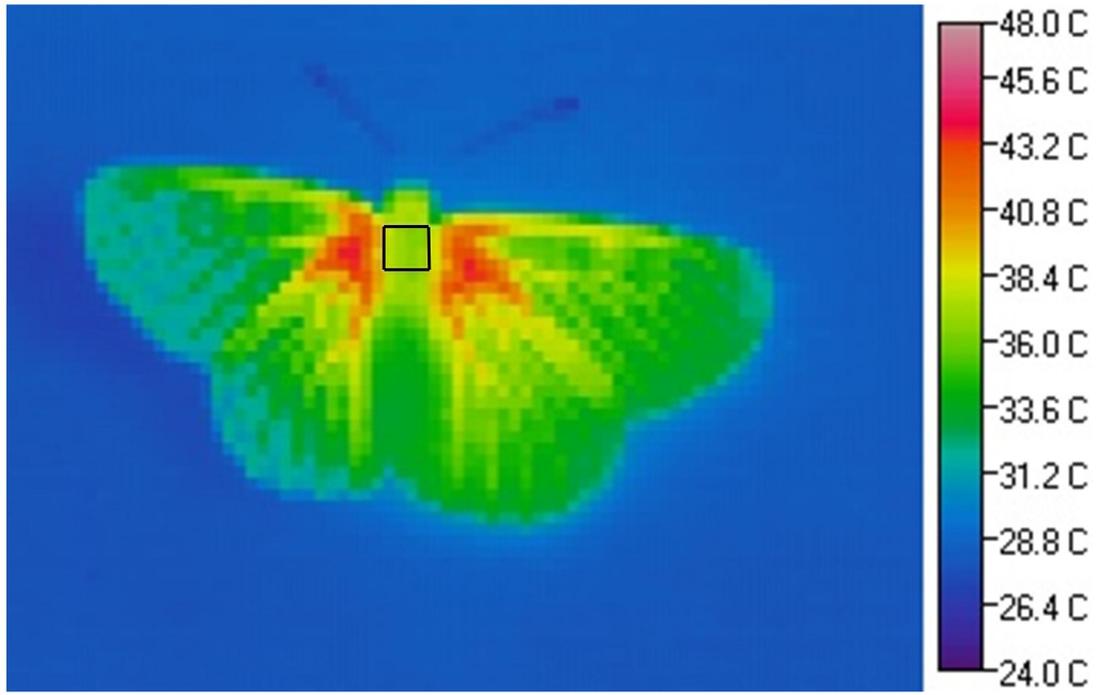
603 Supplemental Table 1: Comparisons between the internal temperature of the butterfly (recorded
604 by a temperature probe), and the external temperature (recorded by a thermal camera), as the
605 butterfly warms up.

606

607 Supplemental Table 2: Calculated significances of inter-region comparisons of takeoff
608 temperature.

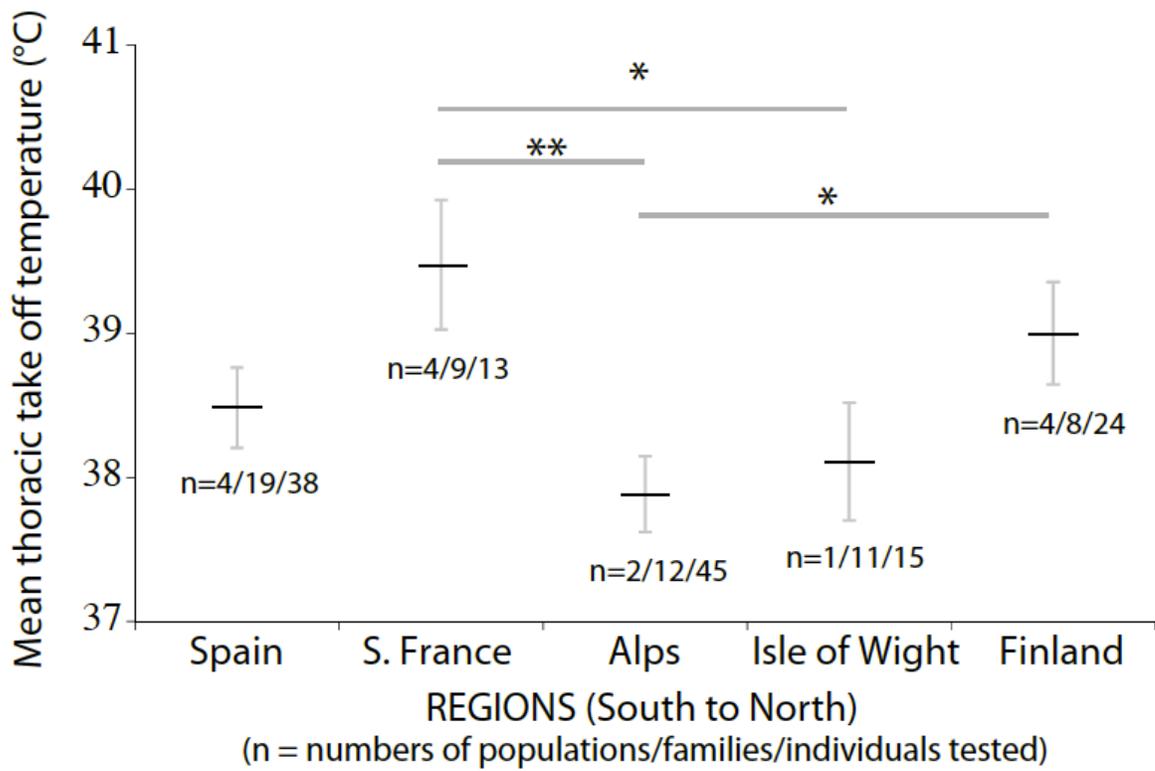
609 Figure 1

610



611

612 Figure 2



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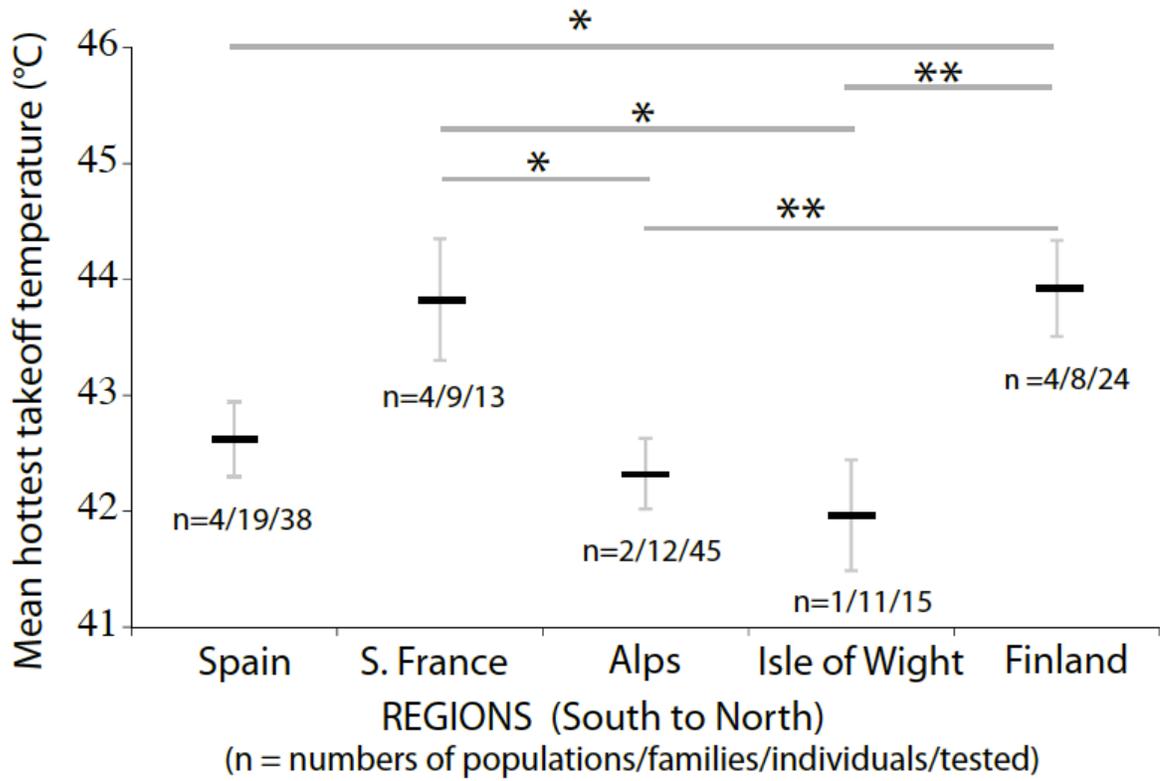
614

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616 Figure 3

617

618



619

620

621 Table 1

Region	Population	Latitude (degrees)	Longitude (degrees)	Elevation (m)	Peak Flight date	Average daytime temperature (°C)	Flight irradiance (w/m ²)	Basking irradiance (w/m ²)
Spain	Sils	41.800	2.730	73	15 May	18.5	1090	1182
	Seva	41.836	2.288	685	20 May	17.2	1101	1185
	Can Terror	41.890	2.707	145	15 May	18.2		
	Sales de Llierca	42.238	2.657	27	1 May [#]	17.6	1058	1186
Southern France	Montpellier	43.580	3.947	3	10 July [*]	25.7		
	Prades	43.725	3.869	77	10 July [*]	25.4	1092	1170
	Cazevielle	43.770	3.825	294	15 July [*]	24.3	1088	1171
	La Pourcaresse	43.771	3.749	278	15 July [*]	24.6		
Alps	Laus de Cervieres	44.856	6.730	1850	20 June	14.8	1102	1183
	Col de Granon	44.963	6.599	2300	30 June	15.3	1101	1184
Isle of Wight	Compton Chine	50.663	-1.478	5	15 June	16.1	1039	1164
Finland	Åland	60.173	19.781	15	25 June	13.9	927	1144

622

623 [#]first generation of two; ^{*} second generation of two. Other populations are almost completely

624 univoltine, with a single generation per year.

Supplemental table 1

Butterfly	Thermal probe temperature (°C)	Average thoracic temperature on thermal image (°C)
1	27.4	29
	30.5	31.3
	33.3	33.3
	35.3	35.3
2	28.6	29.5
	36.7	36.3
3	34.8	34.6
	36.8	37.2
	38.9	38.8
4	27.3	27.7
	31.5	31.5

625

626

627 Supplemental Table 2: Exact probabilities of significant results from statistical analyses of
 628 takeoff temperatures measure as mean thoracic temperature/hottest pixel
 629

	Finland	Isle of Wight	Alps	S France	Spain
Finland		NS/0.004	0.016/0.004	0.032/NS	NS/0.022
Isle of Wight			NS/NS	NS/0.015	NS/NS
Alps				0.004/0.022	NS/NS
S France					NS/NS
Spain					

630
 631